Institut für Lebensmittelsicherheit und -hygiene
der Vetsuisse-Fakultät Universität Zürich

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Arbeit unter wissenschaftlicher Betreuung von
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Characteristics of *Listeria monocytogenes* strains persisting in a meat processing facility over a 4-year period

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

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Tierärztin
Frutigen, Bern

genehmigt auf Antrag von

Prof. Dr. Dr. h.c. Roger Stephan, Referent

2019

Contents

Abstract

Listeria monocytogenes can persist in food production facilities, resulting in serious threats to consumers due to the high mortality associated with Listeriosis, especially in the very young, old and pregnant. We subtyped 124 strains of *L.monocytogenes* isolated from a meat processing facility in Switzerland by serotyping, MLST typing and whole genome sequencing and analyzed their ability to form biofilms and their resistance to the disinfectants benzalkonium chloride (BC) and peracetic acid (PAA). The genotyping results of the strains showed that several clonal populations of *L. monocytogenes* belonging to CC9, CC204 and CC121 persisted in this meat processing facility for at least four years. All of the strains showed biofilm forming capacity comparable to a known high biofilm forming strain. Known efflux pumps for BC were present in CC204, CC9 (*brcABC*) and CC121 (*qacH*) strains, while strains from other CC showed very low MIC's for BC. For PAA, minimal bactericidal concentrations of 1.2 – 1.6 % for 20 min and minimal inhibitory concentrations between 0.1 – 0.2 % were observed. These values were above or in the range of the recommended concentration for use (0.5-1 %), suggesting that PAA might be ineffective at controlling *L. monocytogenes* in this meat processing facility.

Keywords: *Listeria monocytogenes*, meat processing facility, persistence, benzalkonium chloride, peracetic acid, biofilm.

Zusammenfassung

Persistierende *Listeria monocytogenes* Stämme in der Lebensmittelindustrie kommen vor und stellen eine ernstzunehmende Gefahr für die Konsumenten dar. Listeriose ist mit einer hohen Mortalität assoziiert, insbesondere für die Risikogruppe YOPI.

Im Rahmen dieser Studie wurden 124 *L. monocytogenes* Stämme untersucht, welche in einem Schweizer Fleischverarbeitungsbetrieb isoliert und genotypisch mittels Serotypisierung, MLST und Whole Genome Sequencing analysiert wurden. Phänotypisch analysierten wir die Fähigkeit der Stämme Biofilme zu bilden, sowie ihr Resistenzverhalten gegenüber Desinfektionsmittel Peressigsäure und Benzalkoniumchlorid. Genotypisch konnten mehrere persistente klonale Populationen von *L. monozytogenes* in diesem fleischverarbeitenden Betrieb gezeigt werden, nämlich CC9, CC204 und CC121. Alle untersuchten Stämme zeigten im Vergleich zu zwei bekannten Referenzstämmen eine vergleichbar hohe Kapazität einen Biofilm zu bilden. Bekannte Benzalkoniumchlorid Effluxpumpen konnten in CC204, CC9 (*brcABC*) und CC121 (*qacH*) Stämmen nachgewiesen werden. Stämme aus anderen CC zeigten deutlich tiefere MIC's für BC. Für Peressigsäure wurden MBC's zwischen 1.2 -1.6% nach 20 min gezeigt und MIC's zwischen 0.1 – 0.2%. Diese Werte liegen über oder im empfohlenen Bereich für Anwendungskonzentrationen (0.5 -1%). Diese Befunde lassen vermuten, dass Peressigsäure unter Umständen ineffektiv sind um *L. monozytogenes* in fleischverarbeitenden Betriebe zu bekämpfen.

Schlüsselwörter: *Listeria monocytogenes,* fleischverarbeitender Betrieb, Persitenz, Benzalkoniumchlorid, Peressigsäure, Biofilm

Characteristics of *Listeria monocytogenes* strains persisting in a meat processing facility over a 4-year period

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Keywords: *Listeria monocytogenes*, meat processing facility, persistence, benzalkonium chloride, peracetic acid, biofilm..

Paper accepted in: Pathogens

Abstract

Listeria monocytogenes can persist in food production facilities, resulting in serious threats to consumers due to the high mortality associated with listeriosis, especially in the very young, old and pregnant. We subtyped 124 strains of *L. monocytogenes* isolated from a meat processing facility in Switzerland by serotyping, multi locus sequence typing (MLST) typing and whole genome sequencing and analyzed their ability to form biofilms and their resistance to the disinfectants benzalkonium chloride (BC) and peracetic acid (PAA). The genotyping results of the strains showed that several clonal populations of *L. monocytogenes* belonging to CC9, CC204 and CC121 persisted in this meat processing facility for at least four years. All of the strains showed biofilm forming capacity comparable to a known high biofilm forming strain. Known efflux pumps for BC were present in CC204, CC9 (*brcABC*) and CC121 (*qacH*) strains, while strains from other CC showed very low minimal inhibitory concentrations (MIC's) for BC. For PAA, minimal bactericidal concentrations of 1.2 – 1.6 % for 20 min and minimal inhibitory concentrations between 0.1 – 0.2 % were observed. These values were close to or above the recommended concentration for use (0.5-1 %), suggesting that PAA might be ineffective at controlling *L. monocytogenes* in this and potentially other meat processing facilities.

Keywords: *Listeria monocytogenes*, meat processing facility, persistence, benzalkonium chloride, peracetic acid, biofilm.

Introduction

Listeria monocytogenes is a food-borne pathogen that causes disease mainly in vulnerable populations such as very young, old, pregnant or immunocompromised individuals. The high mortality rate of 15-30 deaths per 100 cases of listeriosis [1-4] is mostly attributable to severe central nervous system infections, septicemia, abortions and neonatal listeriosis. In the United States alone, listeriosis is estimated to cause an annual loss of 8800 disease adjusted live years (DALY), of which the majority is due to premature death [5].

Human cases of listeriosis often trace back to food products that were contaminated during production, with subsequent growth of *L. monocytogenes* to high numbers. Ready-to-eat products such as salads or deli meat are of special concern due to the of lack a heating step prior to consumption. The frequent occurrence of *L. monocytogenes* in the environment results in a high probability of introducing the organism into facilities, either on raw materials, through equipment or via employees. Once introduced, several factors increase the probability of a strain being able to establish long-lasting colonization of niches: *L. monocytogenes* has a high tolerance against acid and salt stress, and is able to grow at refrigerating temperature. In addition, the ability to form biofilms may enhance survival, especially in niches that are difficult to reach during cleaning procedures. Further, tolerance against commonly used disinfectants such as the quaternary ammonium chloride compound benzalkonium chloride were observed in *L. monocytogenes* isolates from food processing environments [6-11]. Accordingly, *L. monocytogenes* presents a challenge to the food industry and has been shown to persist in food producing facilities for long times, in some cases for more than a decade. For example, a meat factory in Texas harbored the same strain for at least 12 years, eventually causing an outbreak in 2000 [6,7]. A smoked fish processing plant harbored the same strain of *L. monocytogenes* for 11 years [8]. A study in Ireland found

that seven out of 48 food processors housed a persistent strain, defined as isolated at least six months apart [9].

Here, we analyzed strains from a Swiss deli meat plant where *L. monocytogenes* strains were repeatedly isolated between 2015 and 2018. The aim was (i) to determine whether a clonal population of *L. monocytogenes* persisted in the facility or if *L. monocytogenes* was repeatedly re-introduced, and (ii), to characterize the resistance of the collected strains against benzalkonium chloride and peracetic acid (PAA) and their ability to form biofilms.

Results

Subtyping of 124 Listeria monocytogenes isolated from a Swiss meat plant

A total of 124 strains of *L. monocytogenes* were analyzed in this study. All strains were collected in the framework of a hygiene monitoring program in a meat processing facility in Switzerland between 2015 and 2018 (Table 1). The collection contains 4 strains from 2015, 3 strains from 2016, 32 strains from 2017 and 85 strains from 2018. The samples originated from products (n= 8) and from the food production environment (n=116).

In a first screening of the diversity of the 124 *L. monocytogenes* strains, their serogroup and MLST profile was determined. The majority of the strains (n=73, 58.9%) belonged to serogroup II (containing serotype 1/2c, 3c), 48 strains (38.7%) belonged to serogroup I (1/2a, 3a) and 3 strains (2.4%) belonged to serogroup IV (4b, 4d, 4e). Seven gene MLST revealed that all of the 73 serogroup II strains belonged to CC9 (n=73, 58.9%). The strains belonging to serogroup I were more heterogenous with 31 strains assigned to CC204, seven strains assigned to CC121, four strains to CC20 and to CC29, and one to CC8 and to CC89. The three strains in serogroup IV all belonged to CC6. Only strains from CC9, CC204 and CC121 were consistently isolated over all four years. CC6 strains seem to have been introduced into the facility in July 2017 and persisted until the end of the sampling period in June 2018.

From these results we concluded that it was likely that clonal populations of CC9, CC204, CC121, and CC6 persisted in this facility, while strains from other complexes were sporadically introduced and deemed “presumably non-persistent”. We consciously use the term “presumably” because there is no reasonable way to determine if those strains would have persisted in different environments or under different growth conditions.

In a next step, the genomes of 20 strains were sequenced and a cgMLST comparison was performed (Figure 1). The cgMLST revealed that most strains from the same CC differed in less than 10 alleles; a cut-off for strain clonality [10].

Biofilm formation

The capacity to form biofilms is an important mechanism for persistence [11], and biofilm formation might have contributed to the maintained presence of clonal clusters of *L. monocytogenes* strains in the facility for years. We therefore investigated the biofilm forming ability in a subset of strains of the collective (CC9, CC204, CC121 and CC6) at 22 and 8 °C.

Approximately double the biofilm mass was recovered after 96 h of growth at 22 °C compared to 168 h growth at 8 °C ($p<0.01$) (Figure 2). However, no individual strain had significantly different biofilm formation compared to the other tested strains (supplementary file 1).

At 22 °C, strains from CC9, CC204, CC121 and CC6 showed a biofilm forming capacity in the range of a high biofilm forming (HBF) control strain, and all strains had significantly higher biofilm formation than that of a low biofilm forming (LBF) control strain ($p<0.01$). At 8 °C, all strains, including the HBF control strain were impaired in their biofilm forming capacity and hence, no significant differences between the strains from different complexes, nor between the strains isolated from the meat processing facility vs the control strains were found (Figure 2).

Tolerance to Benzalkonium Chloride

Benzalkonium chloride is a commonly used disinfectant in the food industry and resistance against BC might contribute to the persistence of a strain. One hundred and six out of 124 tested strains were resistant to 10 µg/ml BC (Figure 3). None of the strains, however, was resistant to the cutoff for resistance, 20 µg/ml BC. A cumulative link model [12] revealed that CC29, CC89, CC8 and CC6 had a significantly lower tolerance to BC (combined

average of 4.5 µg/ml BC) compared to the other CC in the dataset (combined average of 9.2 µg/ml BC, $p<0.05$).

A genome-wide search in the 20 sequenced strains revealed the presence of either *brcABC* [13] or *qacH* [14] BC resistance genes in 16 (80%) of the strains (Table 1). *brcABC* genes were present in six strains of CC204 and CC9, respectively. *qacH* was present in four CC121 strains. No strain carried the *emrE* [15] BC resistance gene. All strains that carried resistance genes had the highest measured BC resistance at 10 µg/ml, except ILS AS1-0004 which was resistant to 7.5 µg/ml.

Minimal inhibitory concentration and minimal bactericidal concentration of peracetic acid against L. monocytogenes

PAA is frequently used as a disinfectant in the food production environment with a recommended final concentration between 0.5-1 %. The meat processing facility from which the strains originated used 0.8 % PAA as a sanitizer, and we therefore hypothesized that strains from this collective might have adapted to PAA over time.

The MIC was between 0.1 and 0.2 % for all strains and the MBC was between 1.2 and 1.6 % (Figure 4, supplementary file 1). Since the strains were isolated up to four years apart, these data strongly suggest no adaptation to PAA over time.

All further analyses were performed in a subset of strains that were chosen to represent all four years of collection and were dived over the most frequent clonal complexes CC9 (n = 6), CC204 (n = 4), CC121 (n=3), CC6 (n=2) and CC20 (n=1). To test whether the strains had an unusually high resistance to PAA, a comparison was made to *L. monocytogenes* strains from unrelated sources that belonged to the same clonal complexes [CC9 (n=4), CC204 (n=3), CC121 (n=1) and CC6 (n=1)] (Table 1). The MBCs of the strains was between 1.2 % and 1.6 % with no difference between the clonal complexes, or between the strains isolated from the

meat processing facility versus the control strains (Figure 4a). Therefore, the strains from the meat processing facility showed no increased tolerance to PAA.

To test whether the availability of protein compounds in the test medium affected the outcome, the MBC was compared between PAA in tap water and PAA in BHI. The MBC of PAA in tap water was 0.1 – 0.4 % and therefore lower compared to PAA BHI ($p<0.05$) (Figure 4b), indicating that protein affects the effectiveness of PAA and that therefore there is a protein error for PAA.

We further assessed whether there was an influence of the incubation temperature on the outcome, which would indicate that PAA has a cold error. While MBC values are generally a better indicator for disinfection, we used MIC measurements to address this question because they allow to test the effect of cold exposure over a longer time than MBC measurements. The MIC for PAA was 0.2 % for all strains, regardless of incubation at 4 °C or 37 °C, suggesting no evidence for a cold error for PAA against *L. monocytogenes.*

Discussion

In this study, we have shown that several clonal populations of *L. monocytogenes* persisted in a meat processing facility in Switzerland for at least four years. The persistent strains belonged to CC9, CC204 and CC121. The dominant CC9 and CC121 have previously been shown to be overrepresented in food processing facilities in France [16], in Spain [17], in Switzerland [18], and all over Europe [19]. CC204 on the other hand has only been sporadically isolated from food processing facilities [18] and human patients [20].

Strains from the facility formed biofilm in the range of a high biofilm former. However, no difference was found between the individual strains isolated from the facility, nor between clonal complexes. Subtle differences between clonal complexes might be revealed by replacing the 96-well format biofilm screening protocol used in this study [21] by more

labor-intensive procedures such as culturing biofilms on stainless steel coupons [22] in a follow-up study. Contradictory results have been found by other authors: some studies showed that persistent strains of *L. monocytogenes* form more biofilm than presumed non-persistent strains [11], and persistent strains were more efficient in attaching to surfaces during a short contact time [23]. Others did not observe a difference between persistent vs. presumed non-persistent strains [24], and the ability of *L. monocytogenes* to form true biofilms at all has been critically questioned by some authors [25]. Given the high biofilm forming capacity we found in the strains of *L. monocytogenes* isolated from this meat processing facility, biofilm formation may have contributed to persistence.

The overrepresentation of CC121 in food processing environments has often been explained with their higher resistance to BC due to the presence of the *qacH* or *brcABC* genes that encode efflux pumps [26]. Confirming this, all CC121 strains that were sequenced in this study were positive for *qacH*. Moreover, 80% of all sequenced strains carried either *qacH* or *brcABC*. Given the clonal structure of the CC121, CC9 and CC204 strains in this collection and their uniform resistance to 10 µg/ml BC, it is reasonable to assume that most strains in this collection carry either *qacH* or *brcABC*. However, tolerance to 10 µg/ml BC is below the typical in-use concentrations of BC (500-1000 µg/ml) [27], indicating that BC should be active against the strains in this study.

In contrast, PAA was routinely used for disinfection in the facility at 0.8 % final concentration. Our data show that the MBC (1.2 – 1.6 %) and MIC (0.1 – 0.2 %) values are close to or above this concentration to ensure efficient disinfection. Experiments without nutrients in the medium showed that even in the absence of proteins, the MBC between 0.2 – 0.4 % was still very close to the recommended concentration for use. Under real-life conditions when disinfecting larger areas, it is likely that the concentration of the disinfectant becomes diluted through residual washing water, that residual organic matter is present

and/or that contact times differ due to run-off or delayed reach of hard-to clean niches, which in this case would lead to ineffective concentrations of PAA.

Different strains of *L. monocytogenes* also exhibit high MBC's for PAA in the range of up to 0.5 % [28-31]. In contrast, others concluded that PAA was able to significantly reduce *L. monocytogenes* from multispecies biofilms at 0.15 % [32] and 0.3 % [30], respectively. The MIC of PAA for other organisms seems to be much lower in the range of 0.01 – 0.03% (Gram-positive and Gram-negative flora isolated from citrus fruit) [33], 0.0003 % (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) [34] and 0.0001-0.001 % (*P. aeruginosa*, *S. aureus*, *E. coli*, and *S. epidermidis*) [35]. Given the high tolerance of *L. monocytogenes* against PAA found in our data and by others, the ability of PAA to reduce *L. monocytogenes* from biofilms may have been primarily due to the elimination of the supporting flora in multispecies biofilms.

Our data confirm the absence of adaptation of *L. monocytogenes* to PAA found in other work that found no adaptation over several hundred generations when *L. monocytogenes* were exposed to an industrial disinfectant containing PAA and hydrogen peroxide [36].

Taken together, these experiments show that the concentration of 0.8% PAA used by the facility is insufficient to ensure safe production standards with regard to *L. monocytogenes*. However, PAA is a valuable option for disinfection due to its effectiveness against most bacteria, fungi and viruses, the absence of a cold error, its status as GRAS, and its color- and odorless properties, but only when *L. monocytogenes* is not a major concern. Since PAA at concentrations above 1% is corrosive to equipment and irritating to the eyes via fumes, alternative disinfectant regimen should be considered in food production facilities that struggle to control *L. monocytogenes* in their environment.

Material and methods

Bacterial Strains and Preparation of Bacterial Cultures

The hygiene-monitoring program of the meat-producing facility entailed product samples and swabs of the production environment such as floor drains, trolleys, elevators, cold room floors, scales, production and packaging lines, toilet drains and toilet floors or doors between production sections. All samples were tested for *L. monocytogenes* using the "Assurance Genetic Detection System" (GDS, Biocontrol, Nieuwerkerk aan den IJssel, Netherlands) according to the protocol. In short, swabs or samples were incubated in Half Frazer Broth (HFB, BioRad, Marne-la-Coquette, France) at 30 °C for 48 h, *L. monocytogenes* were further enriched on magnetic beads and then identified via a kit-specific PCR in an "Assurance GDS Rotor-Gene" cycler. To obtain single colonies, the enriched HFB was streaked on Oxoid chromogenic Listeria agar (OCLA) plates (Oxoid, Pratteln, Switzerland) and incubated at 37 °C for 24 h. All strains were kept in 15% glycerol stocks at -80 °C.

To obtain overnight cultures for experiments, the strains were streaked on BHI agar (Oxoid, Pratteln, Switzerland) and incubated overnight at 37 °C. A single colony was inoculated into 5 ml BHI and incubated for 18 h overnight at 37 °C with shaking at 200 rpm in a shaking incubator (Edmund Buehler SM30 / TH30 combination, Huber AG, Reinach, Switzerland). To obtain exponential phase cultures with an OD590 of 0.4, 50 µl of the overnight culture was subcultivated into 5 ml fresh BHI and incubated for 3 h at 37 °C with shaking at 200 rpm.

Serogrouping by qPCR

DNA was isolated from 1 ml overnight cultures using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and eluted in 10 mM Tris pH 7.3. The DNA concentration was measured with a Nanodrop 1000 (Thermo Fisher Scientific, Basel, Switzerland) and PCR templates were standardized to 10^5 copies per 1 µl by dilution in fresh 10 mM Tris. Multiplex

qPCRs were performed according to Vitullo et al. [37] with the following modifications: instead of a triplex-PCR we performed the qPCR as duplex with PUC19 [38] as an internal control on the third channel. Primers and probes were obtained from Sigma-Aldrich (Buchs, Switzerland) according to Vitullo et al. [37] (Table 2) and used at a final concentration of 0.4 µM for the primer and 0.2 µM for the probe.

Cycling conditions for the two-step PCR on a LightCycler 2.0 (Roche Science, Rotkreuz, Switzerland) were the following: 5 s at 95 °C, followed by 40 cycles of 45 s at 95 °C and 45 s at 60 °C.

Multi locus sequence typing (MLST)

MLST was performed on all 124 strains according to Ragon et al. [39]. All primers (Table 2) were ordered from Microsynth (Balgach, Switzerland). Fragment sizes were confirmed by gel-electrophoresis, the products were sequenced by Microsynth (Balgach Switzerland), assembled in Geneious (Version 11.1.4, Biomatters, Newark, NJ, USA) and analyzed using the website of the Institute Pasteur (http://bigsdb.pasteur.fr/listeria/listeria.html).

Whole genome sequencing

Based on the MLST results, a selection of 20 strains (Table 1) were Illumina sequenced. DNA was extracted as for the MLST, sequencing libraries were prepared using the Illumina Nextera DNA Flex chemistry and sequenced on an Illumina MiniSeq (Illlumina, San Diego, CA, USA) with a minimal coverage of 30 x. After quality control with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), the reads were assembled with Spades 3.12.0 [40]. Core-genome multilocus sequence typing (cgMLST) was performed in the software package SeqSphere 4.1.9 (Ridom, Münster, Germany). Assembled genome sequences were imported and blasted against 1701 genes of the seed genome EGD-e using

standard settings [10]. A minimal spanning tree was produced in SeqSphere with the options "ignore missing values pairwise" and "discard genomes with > 3% missing genes". Strains with less than 10 different alleles were considered as belonging to the same complex.

Biofilm formation

Fifteen strains that represented the four clonal complexes that seem to persist in the factory (CC9 (n=9), CC204 (n=2), CC121 (n=2)), (CC6 (n=2)) were chosen to perform biofilm assays with, according to the protocol published by Harvey et al. with minor changes [21]. A single colony was inoculated into 5 ml of tryptone soy broth (TSB, from Fluka, obtained from Sigma-Aldrich, Buchs, Switzerland), incubated for 20 h at 30 °C with shaking at 200 rpm, subcultured 1:250 into fresh TSB, and incubated for an additional 20 h at 30 °C with shaking at 200 rpm. The resulting cultures were adjusted to an OD_{600} of 1.0, diluted 1:80 in TSB and added to 96-well plates, which were incubated for 96 h at 22 °C, or for 168 h at 8 °C, respectively. Biofilms were then washed three times with distilled water, stained with crystal violet, and washed five times with distilled water. The remaining crystal violet was dissolved in ethanol, and the OD_{600} was measured in a Synergy plate reader (BioTek, Lucern, Switzerland). Control strains that were high and low biofilm formers (Institute for Food Safety and Hygiene, Zurich; unpublished results) (Table 1) were included in each experiment.

Tolerance to Benzalkonium Chloride

MICs for BC were determined for all 124 strains included in this study according to Meier et al. with minor changes [26]. Five µl of an exponential phase culture were spotted on BHI plates containing BC at 0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 25.0 and 30.0 µg ml^{-1} (Sigma Aldrich, Buchs, Switzerland). The plates were incubated for 48 h at 37° C. Strains were

considered resistant to the concentration of BC on which confluent growth was observed. The cutoff for resistance was set according to Langsrud et al. [41] as double the value of the lowest concentration that inhibited growth in > 50% of the tested strains.

All sequenced genomes were searched for the *qacH*, *brcABC* and *emrE* BC resistance genes using BLASTP 2.7.1+ [42] with the standard settings and an e-value cutoff of 10^{-20}.

Minimal inhibitory concentration and minimal bactericidal concentration of peracetic acid against *L. monocytogenes*

These assays were performed in PAA diluted in BHI to mimic a worst-case scenario that assumed incomplete cleaning of organic matter from surfaces before disinfection, and to assess the overall effect of PAA on bacteria within an otherwise favorable environment. To determine the influence of the protein in BHI on the outcome, some of the assays were additionally performed in PAA diluted in tap water (City of Zürich, supplementary file 2). First, the MIC and MBC for PAA were determined in a screening of all 124 strains. Given the clonal nature of much of the strain collection, we then compared the resistance to PAA in detail in a subset of the strains: CC9 (n = 6), CC204 (n = 4), CC121 (n=3), CC6 (n=2) and CC20 (n=1). A control dataset contained strains from unrelated sources: CC9 (n=4), CC204 (n=3), CC121 (n=1) and CC6 (n=1) (Table 1).

To obtain the MBC values, serial dilutions of were prepared to obtain final PAA concentrations of 2.8 %, 2.4 %, 2.0 %, 1.6 %, 1.2 %, 0.8 %, 0.4 %, 0.2 %, 0.1 % and 0.05 %. 190 µl of these dilutions were added to 96-well plates and cooled to 4 °C. Then, exponential phase cultures were diluted in 0.9 % NaCl and 10 µl were added to each well to achieve an inoculum of approximately 5x 10^2 CFU/well for the dilution rows in BHI and 5x10^4 CFU/well for the dilution rows in water. The plates were incubated for 20 min at 4 °C. After incubation, the wells were mixed by pipetting and 20 µl were washed in 180 µl 0.9 % NaCl

in a fresh 96-well plate. These plates were centrifuged at 3220 g for 5 min, the supernatant was discarded, and the cells were resuspended in 20 µl 0.9 % NaCl. 10 µl of each well was spotted on the edge of a BHI agar plate and run down the plate by tilting [43]. These plates were incubated for either 7 d at 8 °C to mimic the conditions in a food processing plant or for 48 h at 37 °C to provide more favorable growth conditions. Surviving bacteria were enumerated by direct colony count, and the MBC was defined as the concentration of PAA that produced no colonies. To determine the MIC, the serial dilution plates were incubated at 37 °C for 48 h and at 8 °C for 7 d. The MIC was defined as the concentration of PAA that allowed for no visible growth [44].

Statistical Analysis

All of the experiments were performed in triplicate unless otherwise indicated.

The results were analyzed in R studio version 1.1.456 and all statistical analyses are provided as a supplementary file (supplementary file 1). In short, a linear mixed effects model using lmer in LmerTest [45] was modelled to the biofilm data, and lsmeans was used to create contrasts [46]. A cumulative link model was calculated for the BC and PAA data using polR in MASS [47], and model selection was done with stepAIC in MASS [47]. All graphics were done using ggplot2 [48].

Author contributions

Conceptualization, RS and CG; Methodology, RS and CG; Investigation, AS; Genomics, MS; Writing – Original Draft Preparation, AS.; Writing – Review & Editing, MS and CG; Visualization, AS; Supervision, RS and CG.

Conflict of interest

The authors declare no conflict of interest.

References

1. Barton Behravesh, C.; Jones, T. F.; Vugia, D. J.; Long, C.; Marcus, R.; Smith, K.; Thomas, S.; Zansky, S.; Fullerton, K. E.; Henao, O. L.; Scallan, E. Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996–2005. *J. Infect. Dis.* **2011**, *204*, 263–267.

2. de Valk, H.; Jacquet, C.; Goulet, V.; Vaillant, V.; Perra, A.; Simon, F.; Desenclos, J. C.; Martin, P. Surveillance of *Listeria* infections in Europe. *Euro Surveill.* **2005**, *10*, 251–255.

3. Popovic, I.; Heron, B.; Covacin, C. Listeria: an Australian perspective (2001-2010). *Foodborne Pathogens and Disease* **2014**, *11*, 425–432.

4. Werber, D.; Hille, K.; Frank, C.; Dehnert, M.; Altmann, D.; Müller-Nordhorn, J.; Koch, J.; Stark, K. Years of potential life lost for six major enteric pathogens, Germany, 2004–2008. *Epidemiol. Infect.* **2012**, *141*, 961–968.

5. Scallan, E.; Hoekstra, R. M.; Mahon, B. E.; Jones, T. F.; Griffin, P. M. An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiol. Infect.* **2015**, *143*, 2795–2804.

6. Olsen, S. J.; Patrick, M.; Hunter, S. B.; Reddy, V.; Kornstein, L.; MacKenzie, W. R.; Lane, K.; Bidol, S.; Stoltman, G. A.; Frye, D. M.; Lee, I.; Hurd, S.; Jones, T. F.; LaPorte, T. N.; Dewitt, W.; Graves, L.; Wiedmann, M.; Schoonmaker-Bopp, D. J.; Huang, A. J.; Vincent, C.; Bugenhagen, A.; Corby, J.; Carloni, E. R.; Holcomb, M. E.; Woron, R. F.; Zansky, S. M.; Dowdle, G.; Smith, F.; Ahrabi-Fard, S.; Ong, A. R.; Tucker, N.; Hynes, N. A.; Mead, P. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin. Infect. Dis.* **2005**, *40*, 962–967.

7. Orsi, R. H.; Borowsky, M. L.; Lauer, P.; Young, S. K.; Nusbaum, C.; Galagan, J. E.; Birren, B. W.; Ivy, R. A.; Sun, Q.; Graves, L. M.; Swaminathan, B.; Wiedmann, M. Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment. *BMC Genomics* **2008**, *9*, 539.

8. Vongkamjan, K.; Roof, S.; Stasiewicz, M. J.; Wiedmann, M. Persistent *Listeria monocytogenes* subtypes isolated from a smoked fish processing facility included both phage susceptible and resistant isolates. *Food Microbiol* **2013**, *35*, 38–48.

9. Leong, D.; Alvarez-Ordóñez, A.; Jordan, K. Monitoring occurrence and persistence of *Listeria monocytogenes* in foods and food processing environments in the Republic of Ireland. *Front. Microbiol.* **2014**, *5*, 436.

10. Ruppitsch, W.; Pietzka, A.; Prior, K.; Bletz, S.; Fernandez, H. L.; Allerberger, F.; Harmsen, D.; Mellmann, A. Defining and Evaluating a Core Genome Multilocus Sequence Typing Scheme for Whole-Genome Sequence-Based Typing of *Listeria monocytogenes*. *J. Clin. Microbol.* **2015**, *53*, 2869–2876.

11. Borucki, M. K.; Peppin, J. D.; White, D.; Loge, F.; Call, D. R. Variation in Biofilm Formation among Strains of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* **2003**, *69*, 7336–7342.

12. Agresti, A. *Categorical Data Analysis*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2002.

13. Elhanafi, D.; Dutta, V.; Kathariou, S. Genetic characterization of plasmid-associated benzalkonium chloride resistance determinants in a *Listeria monocytogenes* strain from the 1998-1999 outbreak. *Appl. Environ. Microbiol* **2010**, *76*, 8231–8238.

14. Muller, A.; Rychli, K.; Muhterem-Uyar, M.; Zaiser, A.; Stessl, B.; Guinane, C. M.; Cotter, P. D.; Wagner, M.; Schmitz-Esser, S. Tn6188 - a novel transposon in *Listeria monocytogenes* responsible for tolerance to benzalkonium chloride. *PLoS ONE* **2013**, *8*, e76835.

15. Kovacevic, J.; Ziegler, J.; Walecka-Zacharska, E.; Reimer, A.; Kitts, D. D.; Gilmour, M. W. Tolerance of *Listeria monocytogenes* to Quaternary Ammonium Sanitizers Is Mediated by a Novel Efflux Pump Encoded by emrE. *Appl. Environ. Microbiol* **2015**, *82*, 939–953.

16. Maury, M. M.; Tsai, Y.-H.; Charlier, C.; Touchon, M.; Chenal-Francisque, V.; Leclercq, A.; Criscuolo, A.; Gaultier, C.; Roussel, S.; Brisabois, A.; Disson, O.; Rocha, E. P. C.; Brisse, S.; Lecuit, M. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. *Nature Genetics* **2016**, *48*, 308–313.

17. Martín, B.; Perich, A.; Gómez, D.; Yangüela, J.; Rodríguez, A.; Garriga, M.; Aymerich, T. Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiology* **2014**, *44*, 119–127.

18. Ebner, R.; Stephan, R.; Althaus, D.; Brisse, S.; Maury, M.; Tasara, T. Phenotypic and genotypic characteristics of *Listeria monocytogenes* strains isolated during 2011–2014 from different food matrices in Switzerland. *Food Control* **2015**, 321–326.

19. Nielsen, E. M.; Björkman, J. T.; Kiil, K.; Grant, K.; Dallman, T.; Painset, A.; Amar, C.; Roussel, S.; Guillier, L.; Félix, B.; Rotariu, O.; Perez Reche, F.; Forbes, K.; Strachan, N. Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 3, the comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS) analysis. *EFSA Supporting Publications* **2017**, *14*, 539.

20. Althaus, D.; Lehner, A.; Brisse, S.; Maury, M.; Tasara, T.; Stephan, R. Characterization of *Listeria monocytogenes* Strains Isolated During 2011–2013 from Human Infections in Switzerland. *Foodborne Path. Dis.* **2014**, *11*, 753–758.

21. Harvey, J.; Keenan, K. P.; Gilmour, A. Assessing biofilm formation by *Listeria monocytogenes* strains. *Food Microbiology* **2007**, *24*, 380–392.

22. Heir, E.; Møretrø, T.; Simensen, A.; Langsrud, S. *Listeria monocytogenes* strains show large variations in competitive growth in mixed culture biofilms and suspensions with bacteria from food processing environments. *International Journal of Food Microbiology* **2018**, *275*, 46–55.

23. Lundén, J. M.; Miettinen, M. K.; Autio, T. J.; Korkeala, H. J. Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. *J. Food Prot.* **2000**, *63*, 1204–1207.

24. Djordjevic, D.; Wiedmann, M.; McLandsborough, L. A. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Appl. Environ. Microbiol* **2002**, *68*, 2950–2958.

25. Ferreira, V.; Wiedmann, M.; Teixeira, P.; Stasiewicz, M. J. *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *J. Food Prot.* **2014**, *77*, 150–170.

26. Meier, A. B.; Guldimann, C.; Markkula, A.; Pöntinen, A.; Korkeala, H.; Tasara, T. Comparative Phenotypic and Genotypic Analysis of Swiss and Finnish *Listeria monocytogenes* Isolates with Respect to Benzalkonium Chloride Resistance. *Front. Microbiol.* **2017**, *8*, 397.

27. Hegstad, K.; Langsrud, S.; Lunestad, B. T.; Scheie, A. A.; Sunde, M.; Yazdankhah, S. P. Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health? *Microb. Drug Resist.* **2010**, *16*, 91–104.

28. Poimenidou, S. V.; Dalmasso, M.; Papadimitriou, K.; Fox, E. M.; Skandamis, P. N.; Jordan, K. Virulence Gene Sequencing Highlights Similarities and Differences in Sequences in *Listeria monocytogenes* Serotype 1/2a and 4b Strains of Clinical and Food Origin From 3 Different Geographic Locations. *Front. Microbiol.* **2018**, *9*, 1103.

29. Skowron, K.; Hulisz, K.; Gryń, G.; Olszewska, H.; Wiktorczyk, N.; Paluszak, Z. Comparison of selected disinfectants efficiency against *Listeria monocytogenes* biofilm formed on various surfaces. *International Microbiology* **2018**, *21*, 23–33.

30. Oxaran, V.; Dittmann, K. K.; Lee, S. H. I.; Chaul, L. T.; Fernandes de Oliveira, C. A.; Corassin, C. H.; Alves, V. F.; De Martinis, E. C. P.; Gram, L. Behavior of Foodborne Pathogens *Listeria monocytogenes* and *Staphylococcus aureus* in Mixed-Species Biofilms Exposed to Biocides. *Applied and Environmental Microbiology* **2018**, *84*, 627.

31. Saá Ibusquiza, P.; Herrera, J. J. R.; Cabo, M. L. Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by *Listeria monocytogenes*. *Food Microbiology* **2011**, *28*, 418–425.

32. da Silva Fernandes, M.; Kabuki, D. Y.; Kuaye, A. Y. Behavior of *Listeria monocytogenes* in a multi-species biofilm with *Enterococcus faecalis* and *Enterococcus faecium* and control through sanitation procedures. *Int. J. Food Microbiol.* **2015**, *200*, 5–12.

33. Winniczuk, P. P.; Parish, M. E. Minimum inhibitory concentrations of antimicrobials against micro-organisms related to citrus juice. *Food Microbiology* **1997**, *14*, 373–381.

34. Alasri, A.; Roques, C.; Michel, G.; Cabassud, C.; Aptel, P. Bactericidal properties of peracetic acid and hydrogen peroxide, alone and in combination, and chlorine and formaldehyde against bacterial water strains. *Can. J. Microbiol.* **1992**, *38*, 635–642.

35. Vieira, R. G. L.; Moraes, T. D. S.; Silva, L. de O.; Bianchi, T. C.; Veneziani, R. C. S.; Ambrósio, S. R.; Bastos, J. K.; Pires, R. H.; Martins, C. H. G. In vitro studies of the antibacterial activity of Copaifera spp. oleoresins, sodium hypochlorite, and peracetic acid against clinical and environmental isolates recovered from a hemodialysis unit. *Antimicrob Resist Infect Control* **2018**, *7*, 14.

36. Kastbjerg, V. G.; Gram, L. Industrial disinfectants do not select for resistance in *Listeria monocytogenes* following long term exposure. *Int. J. Food Microbiol.* **2012**, *160*, 11–15.

37. Vitullo, M.; Grant, K. A.; Sammarco, M. L.; Tamburro, M.; Ripabelli, G.; Amar, C. F. L. Real-time PCRs assay for serogrouping *Listeria monocytogenes* and differentiation from other *Listeria* spp. *Mol. Cell. Probes* **2013**, *27*, 68–70.

38. Yanisch-Perron, C.; Vieira, J.; Messing, J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mpl8 and pUC19 vectors. *Gene* **1985**, *33*, 103–119.

39. Ragon, M.; Wirth, T.; Hollandt, F.; Lavenir, R.; Lecuit, M.; Le, M. A.; Brisse, S. A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathog* **2008**, *4*, e1000146.

40. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A. A.; Dvorkin, M.; Kulikov, A. S.; Lesin, V. M.; Nikolenko, S. I.; Pham, S.; Prjibelski, A. D.; Pyshkin, A. V.; Sirotkin, A. V.; Vyahhi, N.; Tesler, G.; Alekseyev, M. A.; Pevzner, P. A. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477.

41. Langsrud, S.; Sidhu, M. S.; Heir, E.; Holck, A. Bacterial disinfectant resistance—a challenge for the food industry. *Int. J. Biodeter. Biodegrad.* **2003**, *51*, 283–290.

42. Altschul, S. F.; Madden, T. L.; Schäffer, A. A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D. J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **1997**, *25*, 3389–3402.

43. *Listeria monocytogenes*; Jordan, K.; Fox, E. M.; Wagner, M., Eds.; Springer New York: New York, NY, 2014; Vol. 1157.

44. Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols* **2008**, *3*, 163–175.

45. Kuznetsova, A.; Bruun Brockhoff, P.; Bojesen Christensen, H. lmerTest: Tests in Linear Mixed Effects Models. *https://CRAN. R-project. org/package=lmerTest* **2016**.

46. Lenth, R. V. Least-Squares Means: The RPackage lsmeans. *Journal of Statistical Software* **2016**, *69*, 1–33.

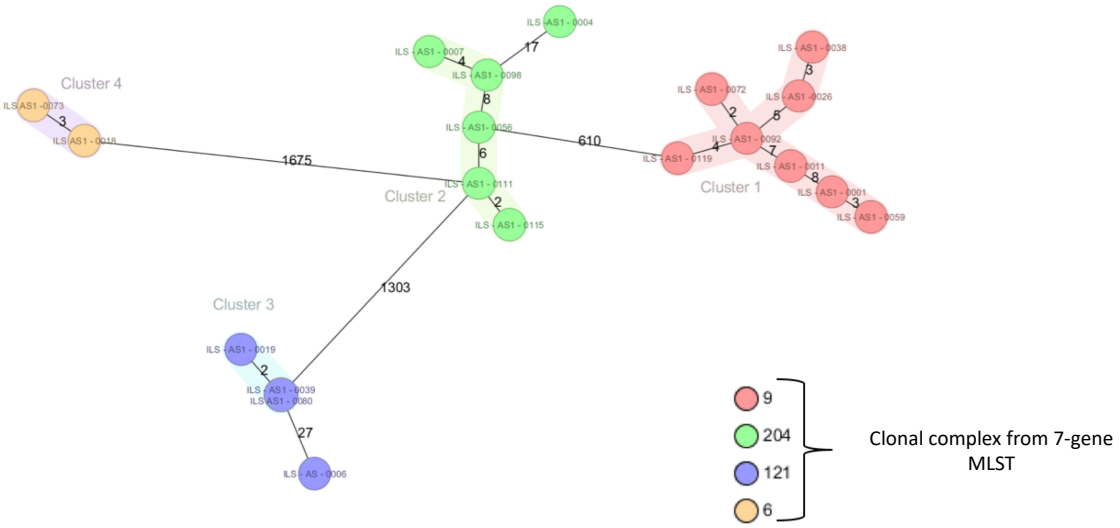
47. Venable, W. N.; Ripley, B. D. *Modern Applied Statistics with S.*; 4 ed.; Springer, 2002.

48. Wickham, H. ggplot2: elegant graphics for data analysis. In *ggplot2: elegant graphics for data analysis.*; Springer, New York, 2009.

Tables & Figures

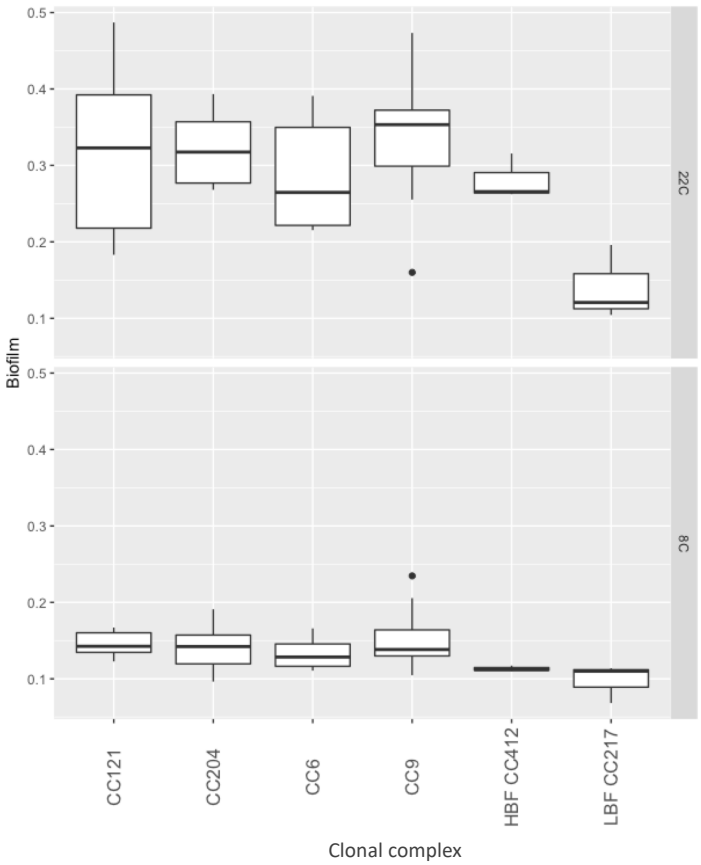
Figure 1

Figure 1



cgMLST for 20 selected strains from the collection. See main text for details.

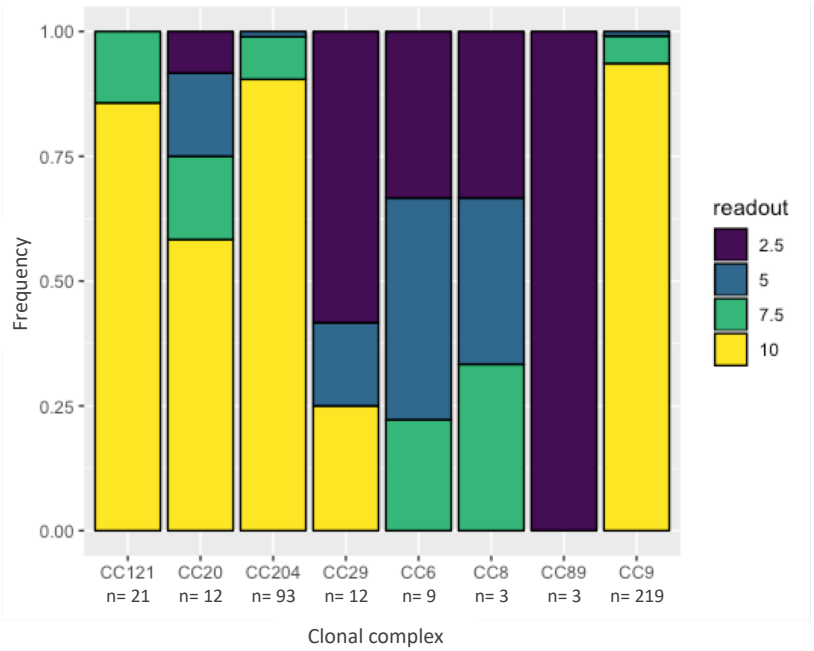
Figure 2



Biofilm formation by clonal complex, at 22 °C and 8 °C. The y-axis represents optical density in a crystal violet assay, the x-axis represents strains by clonal complex.

Figure 3

Figure 3



MIC of BC by clonal complex. The y-axis represents frequency. The colors represent the highest concentration of BC (in µg/ml) at which confluent growth was observed after 48 h at 37 °C. The x-axis represents the different clonal complexes. n= the number of data points that were observed for each clonal complex.

Figure 4

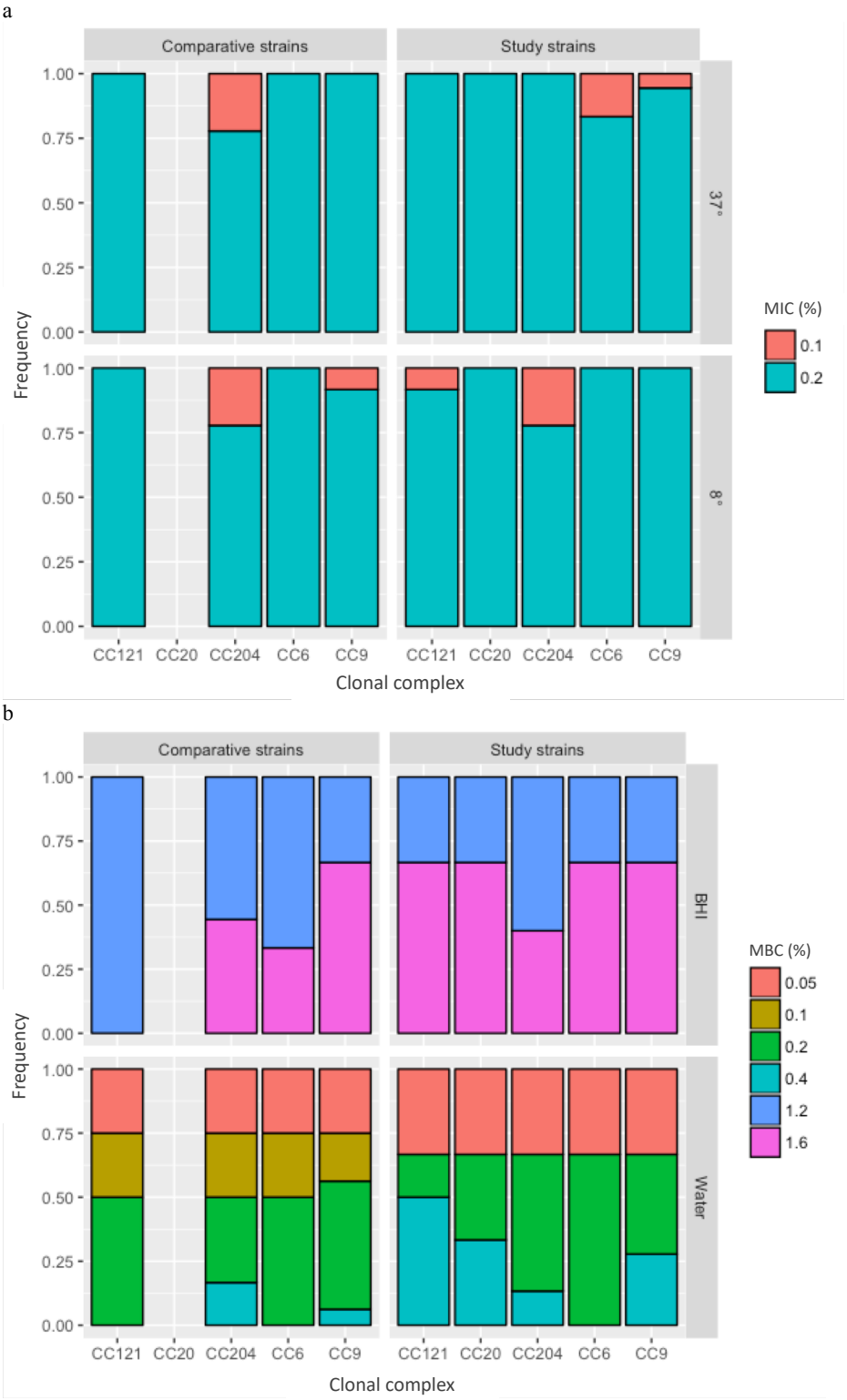


Figure 4. Tolerance to PAA. The x-axis represents the clonal complexes, the y-axis frequency. "Study strains" represent strains isolated from the food processing facility, "Comparative strains" represent strains from the same CC but isolated from unrelated sources. (a) MIC for PAA in BHI, at 37 °C and 8 °C. (b) MBC for PAA by medium, BHI = brain heart infusion. Water = tap water.

Table 1: *L. monocytogenes* strains used in this study (in the final layout, this table should span 2 pages)

Strain	Isolation date	Isolation source	Purpose	Serogroup	Sequence Type	Clonal complex	WGS Cluster	*brcABC*	*qacH*	*emrE*	Lineage
ILS AS1-0001	15.10.15	Food processing environment	Study strain	2	9	9	1	absent	absent	absent	II
ILS AS1-0002	19.10.15	Food product	Study strain	1	121	121		n/a	n/a	n/a	II
ILS AS1-0003	09.11.15	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0004	14.11.15	Food processing environment	Study strain	1	204	204	2*	present	absent	absent	II
ILS AS1-0005	02.02.16	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0006	09.03.16	Food processing environment	Study strain	1	121	121	3*	absent	present	absent	II
ILS AS1-0007	20.10.16	Food product	Study strain	1	204	204	2	present	absent	absent	II
ILS AS1-0008	09.01.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0009	09.01.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0010	09.01.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0011	09.01.17	Food processing environment	Study strain	2	9	9	1	present	absent	absent	II
ILS AS1-0012	09.01.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0013	09.01.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0014	09.01.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0015	26.06.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0016	29.06.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0017	07.07.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0018	07.07.17	Food processing environment	Study strain	4	6	6	4	absent	absent	absent	I
ILS AS1-0019	10.08.17	Food product	Study strain	1	121	121	3	absent	present	absent	II
ILS AS1-0020	12.09.17	Food product	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0021	13.09.17	Food product	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0022	18.09.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0023	18.09.17	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0024	06.11.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0025	06.11.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0026	13.11.17	Food processing environment	Study strain	1	204	204	1	absent	absent	absent	II
ILS AS1-0027	23.11.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0028	05.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0029	05.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0030	05.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0031	05.12.17	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0032	07.12.17	Food processing environment	Study strain	1	20	20		n/a	n/a	n/a	II
ILS AS1-0033	07.12.17	Food processing environment	Study strain	1	20	20		n/a	n/a	n/a	II
ILS AS1-0034	07.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0035	05.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0036	05.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0037	05.12.17	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0038	05.12.17	Food processing environment	Study strain	2	9	9	1	absent	absent	absent	II
ILS AS1-0039	14.12.17	Food processing environment	Study strain	1	121	121	3	absent	present	absent	II
ILS AS1-0040	23.01.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0041	23.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0042	23.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0043	23.01.18	Food processing environment	Study strain	1	29	29		n/a	n/a	n/a	II
ILS AS1-0044	23.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0045	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0046	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0047	25.01.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0048	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0049	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0050	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0051	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0052	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0053	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0054	25.01.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0055	25.01.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0056	25.01.18	Food processing environment	Study strain	1	204	204	2	present	absent	absent	II
ILS AS1-0057	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0058	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0059	25.01.18	Food processing environment	Study strain	2	9	9	1	present	absent	absent	II
ILS AS1-0060	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0061	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0062	26.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0063	26.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0064	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0065	25.01.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0066	25.01.18	Food processing environment	Study strain	1	391	89		n/a	n/a	n/a	II
ILS AS1-0067	06.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0068	06.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0069	07.02.18	Food processing environment	Study strain	1	29	29		n/a	n/a	n/a	II
ILS AS1-0070	06.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0071	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0072	09.02.18	Food processing environment	Study strain	2	9	9	1	present	absent	absent	II
ILS AS1-0073	09.02.18	Food processing environment	Study strain	4	6	6	4	absent	absent	absent	I
ILS AS1-0074	09.02.18	Food processing environment	Study strain	1	29	29		n/a	n/a	n/a	II
ILS AS1-0075	09.02.18	Food processing environment	Study strain	1	8	8		n/a	n/a	n/a	II
ILS AS1-0076	09.02.18	Food processing environment	Study strain	1	20	20		n/a	n/a	n/a	II
ILS AS1-0077	09.02.18	Food processing environment	Study strain	1	29	29		n/a	n/a	n/a	II
ILS AS1-0078	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0079	09.02.18	Food processing environment	Study strain	1	121	121		n/a	n/a	n/a	II
ILS AS1-0080	09.02.18	Food processing environment	Study strain	1	121	121	3	absent	present	absent	II
ILS AS1-0081	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0082	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0083	09.02.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
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ILS AS1-0086	09.02.18	Food processing environment	Study strain	1	20	20		n/a	n/a	n/a	II
ILS AS1-0087	09.02.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0088	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0089	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0090	09.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0091	10.02.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0092	13.02.18	Food processing environment	Study strain	2	9	9	1	present	absent	absent	II
ILS AS1-0093	21.03.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0094	05.04.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0095	05.04.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0096	05.04.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0097	05.04.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0098	22.05.18	Food processing environment	Study strain	1	204	204	2	present	absent	absent	II
ILS AS1-0099	22.05.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0100	21.03.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0101	25.05.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0102	25.05.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0103	25.05.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0104	25.05.18	Food processing environment	Study strain	2	9	9		present	absent	absent	II
ILS AS1-0105	25.05.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0106	25.05.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0107	25.05.18	Food processing environment	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0108	25.05.18	Food processing environment	Study strain	4	6	6		n/a	n/a	n/a	I
ILS AS1-0109	19.06.18	Food product	Study strain	2	9	9		n/a	n/a	n/a	II
ILS AS1-0110	25.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0111	25.06.18	Food processing environment	Study strain	1	204	204	2	present	absent	absent	II
ILS AS1-0112	26.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0113	26.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0114	26.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0115	26.06.18	Food processing environment	Study strain	1	204	204	2	present	absent	absent	II
ILS AS1-0116	26.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0117	26.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0118	26.06.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0119	25.06.28	Food product	Study strain	2	9	9	1	present	absent	absent	II
ILS AS1-0120	16.07.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0121	16.07.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0122	16.07.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS AS1-0123	03.08.18	Food product	Study strain	1	121	121		n/a	n/a	n/a	II
ILS AS1-0124	09.08.18	Food processing environment	Study strain	1	204	204		n/a	n/a	n/a	II
ILS-AS-R-001			PAA reference		204	204		n/a	n/a	n/a	II
ILS-AS-R-002			PAA reference		204	204		n/a	n/a	n/a	II
ILS-AS-R-003			PAA reference		204	204		n/a	n/a	n/a	II
ILS-AS-R-004			PAA reference		9	9		n/a	n/a	n/a	II
ILS-AS-R-005			PAA reference		9	9		n/a	n/a	n/a	II
ILS-AS-R-006			PAA reference		9	9		n/a	n/a	n/a	II
ILS-AS-R-007			PAA reference		9	9		n/a	n/a	n/a	II
ILS-AS-R-008			PAA reference		121	121		n/a	n/a	n/a	II
ILS-AS-R-009			PAA reference		6	6		n/a	n/a	n/a	I
N586			Biofilm reference (LBF)	3a°	412	412		n/a	n/a	n/a	II
N11-1850			Biofilm reference (HBF)	4b°	1290	217		n/a	n/a	n/a	I

* indicates strains that are associated with a cgMLST cluster without being properly in it
° these strains were serotyped with antibodies from Denka-Seiken (Basel, Switzerland)
n/a = not applicable; LBF = low biofilm former; HBF = high biofilm former

Table 2: Nucleotides used in this study (in the final layout, this table should span two columns)

Serogrouping						
Gene	**Primer forward**	**Sequence**	**Primer reverse**	**Sequence**	**Probe**	**chanel**
lmo0737	lmo0737F	GCATCTTGTTTAGCAAGTGGATC	lmo0737R	GAGCACGGAAGTTGCTAGGT	[5'] YY-CCAACACTTTCTCATCAATACCATCTTCCC- [3'] BHQ1	560nm
lmo1118	lmo1118F	CTTAGTATTCCAGGATTTAAGACC	lmo1118R	CCAAAGAACCAAATTGATCGAATC	[5'] FAM -CCTTTATCTTCTCCTGAGTGTATACGCCTC – [3'] BHQ1	510nm
ORF2110	ORF2110F	CACTAATCTCATCGACTATAAACTC	ORR2110R	TGCACAAGCAGCAGCAGAGGAAG	[5'] YY-TCTCCGTCATTTGTTACCGTTTCCCCAAC – [3'] BHQ1	560nm
ORF2819	ORF2819F	ATCACTAAAGCCTCCCATTGAG	ORR2819R	GGAAGATTTCCACGCAATACTC	[5'] FAM- CTCGTAAGATCGATATACGTCATGGCAGTTTCC - [3'] BHQ1	510nm
plcA	plcAF	CGGCGGCACCTAACCAAGTAA	plcAR	CAGTCTGGACAATCTCTTTGAATTTT	[5'] YY-TCAAGATGACTACAATGGTCCGAGTGTGAAAA - [3'] BHQ1	560nm
prs	prsF	CAGGRTTACTCGTTGATTGAATAAC	prsR	GCTGAAGAGATTGCGAAAGAAG	[5'] FAM- CATGACAACCACGGATACTTTCTTCAATGTTAATTTG - [3'] BHQ1	510nm
pUC19	pUCF	GCA GCC ACT GGT AAC AGG AT	pUCR	GCA GAG CGC AGA TAC CAA AT	[5'] ROX- AGAGCGAGGTATGTAGGCG [3'] BHQ2	610nm
MLST						
Gene	**Primer forward**	**Sequence**	**Primer reverse**	**Sequence**		
abc	abcOF	GTTTTCCCAGTCACGACGTTGTATCGCTGCTGCCACTTTTATCCA	abcOR	TTGTGAGCGGATAACAATTTCTCAAGGTCGCCGTTTAGAG		
bglA	bglAOF	GTTTTCCCAGTCACGACGTTGTAGCCGACTTTTTATGGGGTGGAG	bglAOR	TTGTGAGCGGATAACAATTTCCGATTAAATACGGTGCGGACATA		
cat	catOF	GTTTTCCCAGTCACGACGTTGTAATTGGCGCATTTTGATAGAGA	catOR	TTGTGAGCGGATAACAATTTCAGATTGACGATTCCTGCTTTTG		
dapE	dapEOF	GTTTTCCCAGTCACGACGTTGTACGACTAATGGGCATGAAGAACAA	dapEOR	TTGTGAGCGGATAACAATTTCATCGAACTATGGGCATTTTTACC		
dat	datOF	GTTTTCCCAGTCACGACGTTGTAGAAAGAGAAGATGCCACAGTTGA	datOR	TTGTGAGCGGATAACAATTTCTGCGTCCATAATACACCATCTTT		
ldh	ldhOF	GTTTTCCCAGTCACGACGTTGTAGTATGATTGACATAGATAAAGA	ldhOR	TTGTGAGCGGATAACAATTTCTATAAATGTCGTTCATACCAT		
lhkA	lhkAOF	GTTTTCCCAGTCACGACGTTGTAAGAATGCCAACGACGAAACC	lhkAOR	TTGTGAGCGGATAACAATTTCTGGGAAACATCAGCAATAAAC		
MLST sequencing primer						
	OR	GTT TTC CCA GTC ACG ACG TTG TA				
	OF	TTG TGA GCG GAT AAC AAT TTC				

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